cancer xenografted tumor growth. Monoclonal antibodies have defined normal and tumor-associated antigens in urologic cancers and are expected to be useful in immunodiagnosis and cancer therapy in the near future. (6 Refs.)
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Immunodiagnosis of prostate cancer is at a more advanced stage than that of most other tumors. Two well-known markers, prostatic acid phosphatase and prostate-specific antigen, have been used in the clinical management of patients. Prostate-specific antigen is a more sensitive and reliable marker than prostatic acid phosphatase. Serum prostate-specific antigen is effective in monitoring disease status, predicting recurrence, and detecting residual disease. Prostate-specific antigen is a tool for the histological differential diagnosis of metastatic carcinomas, especially in the identification of metastatic prostate tumor cells in distant organs and in the differentiation of primary prostate carcinoma from poorly differentiated transitional cell carcinoma of the bladder. Few data on biological function are available. Prostatic acid phosphatase functions as a phosphotyrosyl-protein phosphatase and prostate-specific antigen as a protease. Physiological function in the prostate remains to be elucidated. Several of the prostate-specific and prostate-tumor-associated antigens, as well as a putative prostate tumor-specific antigen, as recognized by monoclonal antibodies are available. Clinical evaluation of these potential markers is not yet available. (71 Refs.)
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22

Prostate Cancer–Associated Markers

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When compared with most other tumor systems, immunodiagnosis of prostate cancer is better defined, in a more developed stage, and has a more significant role in the management of patients. This is primarily due to the fact that there are two immunochemically well-defined and clinically fully evaluated tumor markers: prostatic acid phosphatase (PAP) and prostate-specific antigen (PA or PSA). Acid phosphatase activity has been used for over five decades as an aid in serodiagnosis of advanced prostatic cancer [1]. Clinical utilization of this enzyme marker, including its limitations, is well known by urologists and oncologists. Realization of the difficulties in searching for a prostate tumor-specific marker and the focused investigation on a prostate-specific marker, an organ-site and cell-type specific antigen, have paved the way for dramatic advances in immunodiagnosis of prostatic cancer in recent years [2].

The established clinical application of prostate specific antigen and prostatic acid phosphatase as well as the potential of recently reported prostate tumor markers will be the subject of this chapter. Basic chemical and biological characteristics of these clinically important markers are also reviewed.

I. PROSTATE-SPECIFIC ANTIGEN

The existence of prostate-specific antigens has been reported for some time [3,4]. Although the identification of these prostatic-specific components had been indicated in the literature, its clinical potential was not appreciated until 1979 when a prostate-specific antigen, initially abbreviated as PA and now commonly known as PSA (the use of PA or PSA in this chapter follows its citation in the original references), was purified and characterized [5].

A. Molecular Characteristics

Purified PA is a glycoprotein (7% carbohydrate, 93% protein) of molecular weight 33 Kd and isoelectric point 6.9 [5-7]. The carbohydrate content of PA includes common hexoses, hexosamines, and 1% of sialic acid. The complete sequence of 240 amino acid

residues in purified PA is also known, with isoleucine and proline as the N- and C-terminal amino acid, respectively [8]. The possible carbohydrate side chains have been postulated to be on asparagine-45 for N-linkage and on serine-69, threonine-70, and serine-71 for O-linkage [8]. The sequence of oligosaccharides and exact location of monosaccharide-amino acid linkages remain to be determined.

Other characteristics of PA include a Stokes' radius of 24 Å, partial specific volume of 0.73 ml/g, and a sedimentation coefficient of 3.1 S [7]. Immunochemically identical isomeric forms of PA have been reported, probably due to a microheterogeneity of the carbohydrate moiety [9].

PA is a highly immunogenic protein, although it is a comparatively small molecule. Immunization of animals, such as rabbit, goat, mouse, and baboon, with purified PA easily generates polyclonal antisera [5,9]. Upon absorption with femal normal serum and other tissue extracts, these polyclonal antisera are fairly specific for human prostate. Only normal, benign, and malignant prostate glands, and seminal plasma, have been found to contain PA reactive with the antisera. Monoclonal antibodies also can be easily generated. It should be emphasized that PA is not a prostate-tumor-specific antigen (prostate neoantigen), since a similar concentration (per unit of protein) is present in normal, benign hypertrophic and cancerous prostate [10]. Also, biochemical and immunochemical data available have clearly indicated that it is different from and not related to PAP [9, 11].

B. Initial Immunoassay System and Clinical Evaluation

With the use of a rabbit polyclonal anti-PA antiserum reagent, a sensitive and reproducible sandwich-type enzyme immunoassay was developed in 1980 shortly after the report on PA [12]. This assay system, with a sensitivity of 0.10 ng/ml, used CNBr-activated Sepharose 4B conjugated anti-PA IgG as a solid-phase and anti-PA IgG conjugated horseradish peroxidase as the quantitative marker enzyme-linked immunosorbent assay (ELISA). In this initial report, serum PA levels from normal male controls were found to range from <0.10 to 2.6 ng/ml, with a mean of 0.47. No serum PA was detectable (<0.10) from normal females or female patients with cancer. Male patients with cancer of nonprostatic origin were found to show serum PA levels similar to those of normal male controls.

Significant findings from this initial evaluation of serum PA included the detection of elevated PA levels in patients with prostate adenocarcinomas. The mean values for various clinical stages were: 4.8 ng/ml for stage A, 5.0 for stage B, 10.1 for stage C, and 24.2 for stage D. Highly elevated serum PA levels (>50 times the normal range) were found in many patients with stages C and D prostate cancer. In addition, patients with benign prostatic hyperplasia (BPH) exhibited a mean of 3.4 ng/ml, which is statistically higher than that of normal male controls and patients with nonprostatic cancer. No statistically significant difference was found between stage A and BPH, a suggestive difference was revealed between stage B and BPH, and a highly significant difference was shown between stage C or D and BPH. In comparison with normal controls, stages B, C, and D showed a highly significant difference: a quantitatively different serum PA level was present in patients with prostate cancer from normal men. These results also suggested that PA assay by this ELISA format alone was of limited use as a screening test or for early detection of prostatic cancer, since some degrees of overlap were observed between early prostate carcinoma and BPH.

Shortly after this initial ELISA for serum PA was established, its potential value in monitoring the disease status and treatment response became apparent [13]. In a pilot and double-blind study, a small number of carefully documented and evaluated patients with stage D_2 prostate cancer in whom previous hormonal therapies had failed were randomized to receive adjuvant chemotherapies (National Prostatic Cancer Project Treatment Group). The data revealed an astonishing prognostic value for patients' survival. Regardless of treatment regimen, the survival was found to be inversely and proportionally related to the pretreatment PA levels. An excellent correlation between PA level and clinical status was also noted: PA levels increased as disease progressed, decreased as disease regressed, and remained fluctuating in patients with stable disease.

These two initial and classic clinically significant reports, along with the original report on its restricted prostate tissue specificity, provided the basis for eventual use of PA as the most promising prostate tumor marker available to date [5,12,13]. Since 1980, improved immunoassays, especially with the use of monoclonal antibodies, have become commercially available. The assay kit manufactured by Hybritech Corp. has been approved by the Food an Drug Administration. Many others are awaiting approval.

C. Immunoassay Systems

The assay system developed by Hybritech Corp. is basically a two-site immunoradiometric procedure [14]. Two monoclonal antibodies, each directed against a distinct epitope on PSA molecule, were used in the kit. The first antibody immobilized on plastic beads serves as a "captor" to bind circulating PSA and separate it from other serum proteins and components; the second antibody, labeled with ^{125}I, serves as the "probe" to quantify the amount of the antigen.

The methodology of this two-site immunoradiometric assay has been most extensively evaluated (for review, see 15). In a recent report the detection sensitivity was shown to be 0.1 ng/ml, with assay coefficient variations ranging from 1.3 to 4.9%. A level of 0-2.8 ng/ml was established as a "reference interval" for healthy men [16]. Serum PSA levels greater than the reference interval were found in 50% of patients with stage A prostate cancer, 80% of those with stage B, and 100% for patients with both stages C and D. Follow-up PSA levels for most of the patients after prostatectomy were within 0-0.2 ng/ml. Most patients exhibiting abnormal PSA values were shown to have clinically detectable and residual disease.

In addition to Hybritech's assay, there are at least two other systems commonly available. One is the conventional competitive-inhibition-type polyclonal double-antibody radioimmunoassay, which is also commercially available (e.g., Diagnostic Products Corp., Yang Laboratories) [17,18]. Another is a monoclonal-antibody-based, two-site immunoenzymometric assay, such as that developed by Cetus, but this is not yet available [19]. This assay basically is similar to Hybritech's immunoradiometric assay, however, instead of labeling ^{125}I to the second monoclonal antibody, horseradish peroxidase is labeled and used as the quantifying marker. Evaluation of monoclonal antibody- and polyclonal antibody-based procedures is available, especially Hybritech's monoclonal immunoradiometric assay and Yang's polyclonal based radioimmunoassay. Comparative data for these two assays are available [16]. In a most recent report comparing PSA-Hybritech and PSA-Yang assays, both were reported to detect a similar percentage of abnormal values in patients with cancer of the prostate. However, controls showed a higher percentage abnormal (primarily BPH patients) by PSA-Yang assay (3% Hybri-

tech vs. 17% Yang). Interassay and intra-assay variabilities were also higher with PSA-Yang, as reported previously by others, as was cost per patient. Based upon ease of assay performance, reliability, level of false-positive results, and cost, PSA-Hybritech was reported to be the preferred method [20].

D. Stability of Circulating Prostate-Specific Antigen

Stability of serum PSA as measured by the monoclonal antibody-based immunoradiometric assay of Hybritech has been investigated [21]. As expected, serum PSA is more stable at −20°C or −80°C than at 4°C. Serum specimens containing various levels of PSA (3.7-321.9 ng/ml) were stored at 4°C, −20°C, and −80°C for different periods of time. Aliquots were measured for PSA levels. Results indicated that no significant difference in PSA level was found between initial specimens and last aliquots stored for 7 days at 4°C, or between those stored at −20°C and at −80°C for up to 9 months. Thawing/freezing (at −20°C and −80°C) for eight times over a 6 month period did not affect the PSA level. Acidification of specimens also has no effect on the serum PSA levels. Therefore, with or without acidification, serum PSA is relatively stable and can be reliably quantified within 7 days if stored at 4°C or after long-term storage at −80°C [21]. Serum PSA also is stable for 48 hr at room temperature [22].

E. Serodiagnosis and Staging of Prostate Cancer

Since PSA levels are elevated in some patients with BPH, to eliminate this diagnostic difficulty some investigators have used a higher cutoff point. When an operational limit of upper normal, set at 10 ng/ml, was used, serum PSA levels were found to be elevated in 43% of 91 untreated patients with localized prostate cancer, 92% of 60 untreated patients with advanced disease, and none of 10 patients with BPH. Thus, the use of such an "operational" limit may be an approach to resolving the "false" PSA positive in BPH patients. Of course, ultimate diagnosis should be confirmed with biopsy. Elevation of PSA levels in BPH patients usually was slight and generally would not interfere with clinical interpretation [23]. From a large number of reports available, it is evident that an elevated serum PSA level is found in a great number of patients with an early stage of prostate cancer (10-50%) and in a significant majority of patients with metastatic disease (>90%).

Serum PSA levels appear to be associated with stage of prostate cancer. In a recent study involving 60 patients with prostatic carcinoma, 24 had localized disease (M0) and 36 had metastatic spread (M1) as judged by bone scan. An elevated PSA level (>10 ng/ml) was found in 16 (67%) of those with M0 disease and 34 (95%) of those with M1 disease [24]. However, data from this study revealed that PSA levels reflected neither the histological grade nor the local stage of the tumor, and were of little value in estimating tumor burden. In the study mentioned above involving 74 patients undergoing radical prostatectomy, PSA levels were elevated (>10 ng/ml) in 59% (26/44) with estracapsular disease and in only 7% (2/30) without extracapsular disease, that is, in the 26 of 28 patients (93%) with extracapsular disease [23]. Overall, an elevated PSA level was found in stage B_2 (2/6), C_1 (2/13), C_2 (4/6), C_3 (8/8), and D_2 (12/17). None of the 10 patients with A_1, 4 with A_2, or 10 with B_1 level tumors was found to have a PSA level >10 ng/ml.

F. Monit ring Prostate Cancer

At the present stage of development, measurement of serum PSA is of most clinical value in monitoring treatment response and predicting disease recurrence of patients with cancer as initially reported [13]. In a study of 152 patients, serial serum measurements showed that PSA either reflected or predicted clinical status in more than 97% of the patients. PSA was shown to be of significant prognostic value before and during endocrine therapy of 49 patients with stage D_2 disease, and to be of predictive value in clinical disease in 68 patients with progression and 35 patients with no progression [23].

If one uses 4 ng/ml instead of 10 ng/ml, the significance of PSA levels following radical prostatectomy is also apparent [25]. Preoperative PSA values were correlated with clinical stage and tumor volume in clinical stage A or B patients along with 28 patients with inoperable clinical stage C or D_2, but it could not accurately predict capsular penetration. Postoperative PSA levels were correlated with pathological stage of disease, and frequently associated with tumor recurrence.

A "reference range" of serum PSA levels obtained from 473 normal men in a recent study was reported to be up to 3.9 ng/ml (97.5 percentile), with a mean of 1.02. Age appears to be a factor, since an upper limit of 1.8 was found for 26 younger men (<40 years old) and 5.7 for 208 older men (>40 years old). In this study, a total of 174 serum samples from 80 patients with stage D metastatic prostate carcinoma over a 9 month period were analyzed for the association between disease activity and frequently of PSA elevation. PSA levels consistently correlated with disease status or predicted recurrence before clinical disease was evident [16].

The initial report that serum PSA level can be used to detect residual disease also has been confirmed by many investigators [26,27]. In a recent report PSA was used in the preoperative and postoperative evaluation of localized prostatic cancer treated with radical prostatectomy. Preoperatively, PSA levels were found to correlate directly with capsular penetration, lymph node involvement, and seminal vesicle involvement, although the diagnostic accuracy of an elevated PSA level on an individual basis was only 55% for capsular penetration and 50% for seminal vesicle involvement and lymph node involvement. Postoperatively, 127 patients were followed up for 2 months to 8.6 years, with a mean follow-up of 2 years. Of the 101 paitients who had favorable pathological findings at operation, 91% (15/20 patients with capsular penetration and 77/81 organ-confined cancer) had a follow-up PSA level in the range of 0.0-0.2, whereas only 19% (5/26) with either seminal vesicle involvement or lymph node involvement had a PSA level <0.2 ng/ml. All patients with documented clinical recurrence had an elevated follow-up serum PSA level. Also, the half-life of circulating PSA was calculated to be 3.15 days. PSA can therefore be used to detect residual cancer during the postoperative period.

In another recent study that also involved a large group of controls and patients, the normal range (mean ±2 SD) was found to be 0-2.5 ng/ml from 157 subjects [28]. Age variation in PSA level was not detected in this report using polyclonal radioimmunoassay as that in previous reports using monoclonal radioimmunometric assay [16,24]. For 127 patients with untreated prostate cancer, an elevated PSA level was found in 122 patients: 7/12 of clinical stage A (after transurethral resection), and 115/115 in stages B, C, and D. An unusual number of patients with BPH (63/73) also exhibited elevated levels. PSA level increased with advancing clinical stage. Through a series of multiple regression analysis, PSA was shown to be associated with estimated tumor volume in 45 patients undergoing radical prostatectomy. After the surgery, PSA routinely fell to

undetectable levels, with a half-life of 2.2 days. In 6 patients evaluated postoperatively with serial PSA assays, PSA appeared to be useful in detecting residual and early recurrence of tumor and in monitoring response to radiation therapy [28].

G. Circulating Prostate-Antigen-Binding Globulin

Since PA is a prostate-organ- and epithelial cell-type-specific product, the formation of autoantibodies by the host would be a natural phenomenon according to classic immunology. Using a reverse immunoassay modified from the ELISA for serum PA initially described [12], circulating PA-binding globulin levels were measured in 167 serum specimens, including those of 18 normal men, 14 normal women, 10 male patients each with cancer of the lung, pancreas, and colon-rectum, 25 BPH patients, and 80 patients with various clinical stages of prostate cancer [29]. More than 10 μg/ml of PA-binding globulin was detected in at least 5-10% of the first four groups, even in the sera of normal women, and most profoundly in patients with stage D metastatic prostate carcinoma, in whom markedly elevated PA-binding globulin levels (>50 μg/ml) were detected in more than 50% of patients.

The data from the two initial clinical reports suggest that the presence of this circulating PA-binding globulin does not affect an accurate measurement of serum PA level [12,13]. However, one recent letter from Yang Laboratories has reported, based on very crude data from one single experiment, that the zero diluent in two PSA-Hybritech kits possibly containing a trace of prostate-antigen-binding globulin would interfere with Yang-polyclonal radioimmunoassay kit for PSA [30]. In response, Hybritech has presented data in detail showing otherwise, and indicating additionally that in one PSA-Yang kit tested, two extra ^{125}I-labeled tracers (a major one at 25 KDa and a minor one at 32 KDa), in addition to PSA were detected, and this may be the cause of nonspecific binding observed in the PSA-Yang kit [31].

Despite the possible analytical interference of circulating PA-binding globulin in one radioimmunoassay (RIA) PSA system [32], the potential biological significance of PA-binding globulin should be emphasized. PA-binding globulin isolated from sera of stage D patients was shown to be IgG, in both free form and PA-complexed form [29]. One working hypothesis derived from this finding is that cancer of the prostate is an autoimmune disease (as far as PA/PA binding globulin is concerned), and it raised the possibility of in vitro generation of human monoclonal antibodies directed against PA. Indeed, by fusing a lymphocyte preparation from lymph nodes taken from a patient with early stage of prostate cancer with human lymphoblastoid cells, monoclonal antibodies of human origin reactive with PA have been successfully prepared [33]. This result confirms the hypothesis that PA is an autoantigen of human prostate and that the lymphocytes in patients with prostate cancer are sensitized in vivo.

H. Biological Function

Little information on the biological function of PA is available and its important is yet to be fully appreciated. Purified PA has been shown to exhibit a mild protease activity [34]. This mild enzyme activity of PA examined with various natural and synthetic protease substrates and inhibitors has been characterized as distinct from that of any other known protease.

When the complete amino acid sequence of PA was used and compared structurally with those of the known enzymes, a high degree of sequence homology was observed

with serine proteases of the kallikrein family [8]. "Chymotrypsin-like" and "trypsin-like" activity of PA have been detected. The finding that protease activity of PA could be inhibited by Zn^{++} and spermidine, among others, is most intriguing [8,34]. It is well known that normal prostate contains a relatively high level of Zn, and a reduced Zn level has been reported in malignant prostate. The reduced Zn level may lead to an increased proteolytic activity of PA in prostate tumor. Proteases are also associated with invasive behavior of tumor cells. The exact biological rule, if any, of PA in prostate adenocarcinoma will be of fundamental and clinical interest. Also, spermidine is found in abundant amounts in prostatic fluid, and plays a central role in the growth regulation of cells. A recent report has associated the proteolytic activity of PA with liquefaction of seminal clots and with the structural protein of human seminal coagulum (the primary secretory protein from seminal vesicle) as a possible in vivo physiological substrate [35]. These observations all suggest a potential important role that would have to be played by PA in biology and/or function of the prostate.

One aspect of PA's protease activity may be of interest in a PA assay system, that is, the autohydrolysis of PA, as in autolysis in chymotrypsin. Analysis of the amino-terminal sequence of purified PA has defined the possible localization of three endoproteolytic cleavages: lysine-148, lysine-185, and arginine-85 [8]. Similarly to those of other serine proteases, these cleavages may be autocatalytic. To avoid or minimize this potential autohydrolysis, the addition of protease inhibitors to PA assay reagents or PA specimens should be considered whenever necessary.

I. Immunohistopathology

In addition to being used as an effective serum marker for prostate cancer, PA is a most reliable marker for the immunohistopathological examination of tumors involving the prostate, and for identification of the prostate origin of metastases in various organs and sites. Due to its unique and stable antigenic determinants on the molecule, the expression of PA was preserved in conventionally prepared formalin-fixed, paraffin-embedded surgical and autopsy tissue specimens. The characteristic of PA expression specific for the prostate epithelial cells and in related components makes immunohistochemical staining a simple and effective tool for identifying the distribution of PA in various cells and tissues commonly examined in pathology laboratories.

The technique of immunohistochemistry have been described in detail in other chapters, and will not be repeated here. It should be emphasized once more that the specificity of the primary antibody reagent is the most critical factor in immunohistopathological evaluation. With its defined and restricted expression on prostatic epithelial cells, PA has been found to be the most useful histological marker in the recent history of the immunohistopathology of prostate neoplasms.

The first of such reports was published in 1981, and used a selected rabbit polyclonal antiserum reagent and a large panel of human tissue specimens [36]. In formalin-fixed paraffin-embedded tissue sections, 19 primary prostate carcinomas and 49 metastatic prostate carcinomas all showed positive staining for PA. The intensity of staining reaction was found to vary from cell to cell, from area to area, and from primary to metastatic tumor in the same patient. The positive immunoenzyme stain was confined to cytoplasm of epithelial, with greater concentration of brown granules in the paranuclear area of the luminal aspects of the cells. A most important finding was that none of the 78 nonprostate neoplasms was stained positively for PA. Of particular clinical interest was

that 17 tumors of the urinary bladder with extensions to the prostate gland all stained negatively.

It should be noted that normal and hyperplastic glands of the prostate do express PA. Positive PA stains were found in the epithelial lining of acini and ducts as well as in secretion and concretions. However, the epithelia of periurethral glands, seminal vesicles, vasa deferentia, transitional epithelia of the urinary bladder and prostatic urethra, the glandular lining of von Brunn's nest,and areas of cystitis glandularis were negatively stained for PA. It was also interesting to observe a transition between the positive staining of prostatid ducts and the negative staining of the metaplastic squamous epithelium in prostatic infarcts. All other normal tissues, such as kidney, testis, stomach, liver, colon, rectum, pancreas, lung, breast, and salivary gland were all negative [36].

This report, with an extensive series of normal and pathological tissue specimens, revealed the universal expression of PA in primary and metastatic prostate carcinomas and the consistently negative findings in nonprostatic tumors or other normal tissues. This report also established the initial clinical application of PA in immunohistopathological examinations, providing a simple tool for the differential diagnosis of metastic adenocarcinoma in male subjects (e.g., tumor cells in bone marrow, lymph nodes, and distant organs). It also serves as a tissue marker for the histological classification of tumor involving prostate gland and adjacent tissues (e.g., bladder and rectum) and resolves one of the most common diagnostic problems in pathology.

J. Differential Diagnosis of Bladder from Prostate Carcinomas

This clinical application was best exemplified in a study in which 15 urinary bladder adenocarcinomas (11 in men, 4 in women) and 9 bladder tumors (5 in men, 4 in women) with mixed glandular and transitional features were examined with commercially available polyclonal reagents [37]. None of the tumors seen in either the male or female groups was positively stained for PA, although 3 of the 11 adenocarcinomas and 1 of the 5 tumors with mixed glandular and transitional features in men were positive for PAP, and 2 from each group in women also were positive for PAP. Rare isolated cells in two adenocarcinomas were reported to be slightly stained for PA.

A prospective clinical study involved tissue diagnosis in 21 patients in whom the tumors of the head and neck of the bladder could not be categorized as prostate or urothelial in origin by clinical and endoscopic assessment or by conventional histopathological examination [38]. In 8 of the 21 patients with lesions of the bladder neck, 4 had a past history of prostate tumor, 2 with urothelial tumors, and the other 2 with both. PSA was positive in three of the four patients with a previous prostate neoplasm and in none of the two with a previous history of transitional cell carcinomas. In the two patients who had had both prostatic and transitional cell carcinomas in the past, the bladder neck lesion was PSA-positive in one and negative in the other. All patients with PSA-positive lesions were treated as having prostate carcinomas and all negative ones as transitional cell carcinomas of the bladder. The tumors of the bladder neck occurred de novo in the remaining 13 patients, of which 8 were PSA-positive, indicating a prostate origin, and 7 were treated for prostate carcinoma. In the remaining patient, PSA stain was focal and only weakly positive. He underwent radiotherapy and radical cystoprostatectomy, since the endoscopic appearances and histological results, showing an anaplastic carcinoma, were considered more indicative of urethelial than a prostate tumor. Subsequent examination of the surgical specimens confirmed this to be a prostate tumor. In five patients

the bladder neck tumor was PSA-negative. Four were treated appropriately for uroethlial tumor and one, with history of rectal cancer, was treated for recurrence of rectal carcinoma. In two additional patients, the prostate origin of lymph node metastases with atypical tumor morphology (cervical nodes in one and retroperitoneal nodes in the other) was detected by PSA-positive stain in the absence of a clinically apparent primary. The primary carcinomas were subsequently confirmed on prostate biopsies [38].

K. Identification of Metastatic Prostate Carcinoma

The application of PA to identification of metastatic origin of the prostate was further established with the use of a murine anti-PA monoclonal antibody F5 [39]. The expression of PA was examined in a panel of metastatic tumors, including 25 of prostatic origin and 73 of nonprostate carcinoma origin. Regardless of the site of dissemination or the malignancy grade, all secondary metastases originating from the prostate were positively stained. In contrast, nonprostatic metastases were all negative, including those primary tumors of other genitourinary tracts. Again, such promising clinical results were due primarily to the fine specificity of the F5 monoclonal antibody to the prostate, which had been characterized previously [40].

As commercial reagents become readily available, immunohistochemical staining of PSA has been more commonly practiced in pathology laboratories than in the past. In general, most of the recently published results have confirmed the original findings reported in 1981 [36]. However, several reports on rare and difficult types of prostate neoplasms are of interest. Positive PSA staining has been observed in endometrioid prostate adenocarcinoma and mucinous prostate adenocarcinoma; adenoid cystic carcinoma of the prostate showed negative findings [41-43]. Heterogeneity of small-cell carcinoma of the prostate also can be better appreciated than from morphological examination alone, since adenocarcinoma stained positively for PSA while the small-cell component stained negatively [44].

II. PROSTATIC ACID PHOSPHATASE

The measurement of acid phosphatase has been used for over five decades as an aid in the diagnosis of metastatic prostatic cancer [1]. The uses and limitations of this prostate tumor marker are well known. Several reviews are available for reference [45-48]. Only recent developments in its use will be discussed here. These include the comparison of PAP with PA in serodiagnosis of prostate cancer, in vivo radioimmunodetection of prostate cancer, and biological function of PAP.

A. Prostatic Acid Phosphatase Assay vs. Prostate-Specific Antigen Assay

At the present time, with data available from the extensive clinical evaluation of serum PA assay, it is generally concluded that PA is more sensitive and reliable than PAP as a prostate carcinoma marker. Abundant reports are available documenting the comparative values of PAP vs. PA in prostate cancer. Only a few most recent references are discussed. In a report dealing with comparison of monitoring and staging values between the two markers in patients with regionally confined prostate cancer with extracapsular disease, 93% were shown to have an elevated PSA level (>10 ng/ml), whereas elevated PAP values (>4 ng/ml) were detected in only 59% (using 97th percentile among BPH patients for both assays). In 86 patients with disseminated disease, 98% were documented with an

elevated PSA level while 78% showed elevated PAP levels. Moreover, in 76% of patients who had elevated PSA and PAP levels, the PSA level was more elevated in 94%. Thus, PSA levels were almost always elevated in clinically active metastatic carcinomas of the prostate and were more clearly elevated than PAP in those patients who also had an elevated PAP level [23].

In another report of 127 patients with newly diagnosed prostate cancer, serum PSA level was elevated (>2.5 ng/ml) in 96% including 7 of 12 with stage A and all 115 with stage B–D [28]. In contrast, serum PAP levels were elevated (>2.1 ng/ml) only in 45% of the patients: none in 12 with stage A, 9% in stage B_1, 39% in stage B_2, 40% in stage B_3, 64% in stage C, and 96% in stage D_2. In a prospective comparative study of 80 patients with metastatic stage D cancer, while the overall frequency of abnormal PSA levels was only 76%, the PAP elevation occurred in even fewer patients (60%) [26].

Serial measurements for both markers in 69 patients receiving hormonal therapy were also available. Levels of PSA and PAP reflected the clinical progress in 70% (48/68), PSA alone in 27%, and PAP alone in 1.5%. In 33 patients for whom serial sera were available at least 6 months prior to clinical progression, elevated PSA and PAP were found in 30%, PA alone in 64%, and PAP alone 0% [23].

B. Radioimmunodetection of Prostate Cancer

No report is yet available for PA in in vivo radioimmunodetection of prostate cancer. However, initial evaluation of the use of anti-PAP antibodies has been reported both in animal and humans. The feasibility of using anti-PAP monoclonal antibody in the radioimmunodetection of human prostate carcinoma xenograft has been reported [49]. In a human prostate tumor (LNCaP)-nude mouse model and using ^{125}I-labeled anti-PAP monoclonal antibodies, the tumor/blood localization ration, 7 days after administration of antibodies, was six times higher in the anti-PAP antibody group than in the control groups.

Two previous reports involving a limited number of patients have shown that radioiodine-labeled polyclonal anti-PAP antiserum can be applied to the radioimmunodetection of metastatic prostate cancer [50,51]. A more systematic study has become available most recently, which evaluated 25 patients with stage D_2 disease using ^{111}In-labeled monoclonal antibody (PAY 276) [52]. The patients were evenly classified into five groups. Patients received an infusion of 5, 10, 20, 40, and 80 mg antibody, respectively, in each group, 1 mg of which was labeled with 5 mCi ^{111}In. Imaging was performed at 24, 72, and occasionally 120 hr after infusion. No adverse reactions (skin rash, bronchospasm, tachycardia, febrile reaction, or hypotension) were noted in the patients. No significant changes in serial hemoglobin levels, platelet count, blood chemistry profile, or urinalysis were noted. As with other clinical trials with murine monoclonal antibody, some patients did develop human antimouse antibodies (HAMA; 8 of 16 tested, 6 of whom received 40 mg or more PAY 276).

When compared with conventional radiographs and bone scintigraphy, which were considered in the study to be the definitive indicators of metastatic disease, the monoclonal antibody scan visualized at least 1 lesion in 19 of 25 patients: 4 in groups 1 and 2, and all 15 in groups 3, 4, and 5. Antibody scans detected 7 of 32 metastases in group 3, 31 of 58 in group 4, and 101 of 135 metastases in group 5. An increase in the detection of metastatic lesions was apparent as a greater concentration of unlabeled monoclonal antibody was administered. This increase in detection rate can be attributed to

the decrease in the liver-to-heart distribution ratio of ^{111}In-labeled antibody. This approach to the increase in radioimmunodetection of tumor with the use of "cold" unlabeled monoclonal antibody has been reported with other monoclonal antibodies and tumor systems [53]. Nevertheless, this initial trial with 25 patients with stage D_2 disease does provide encouraging results for future investigations.

C. Biological Function

Although PAP has been known for a long time, its basic characteristics are still relatively unknown. Data available indicate that PAP is a glycoprotein of 100 kD, perhaps in a dimeric form, consisting of 87% peptide and 13% carbohydrate in various isomers (pI 4.8-53.) [54]. Only a partial amino acid sequence with lysine as the N-terminal (arginine in one report) has been determined [55,56]. The PAP molecule contains several antigenic epitopes, all of which are shared in various degrees (from 5% to more than 30%) with other organs [57]. Therefore, it is not as specific for prostate as PA.

Extensive investigations have recently been conducted to explore the biological function of PAP. Preliminary results, as yet few, are of potential interest. Available evidence suggests that, as for the growth rate of the prostate itself, PAP is regulated by androgens. Its physiological function has been suggested recently to be that of phosphotyrosyl-protein phosphatase, since PAP has been shown to exhibit the activity of this enzyme as well, that is, it hydrolyzes or removes the phosphate group linked to tyrosine residue from phosphotyrosine-containing proteins [58,59]. Since tyrosine phosphorylation of proteins is generally believed to play a role in the control of cell proliferation, PAP is thus speculated to act in a reverse role of phosphotyrosine kinase. In addition, epidermal growth factor receptor has been mentioned as the major substrate for tyrosine kinase activity in prostate carcinoma cells [60]. These initial observations and speculations have generated renewed interest in the biological investigation of PAP. We hope that gene cloning and other molecular biological tools may provide a clearer insight into the role of PAP in the physiology of the prostate.

III. MULTIPLE MARKERS IN IMMUNODIAGNOSIS OF PROSTATE CANCER

A. Prostate-Specific Antigen and Prostatic Acid Phosphatase

Based on the working hypothesis that PA and PAP are two distinct biochemical and immunological secretory products, a combination of these two markers may provide increased specificity and/or sensitivity in the serodiagnosis of prostate carcinoma. One approach would be to adjust the upper normal range in both assays to eliminate the BPH specimens as much as possible. An initial evaluation reported a few years ago did produce such an additive clinical value when both markers were assayed simultaneously [61]. In this study, serum specimens from 22 normal healthy men, 30 patients with advanced nonprostate adenocarcinomas, 29 patients with BPH, and 192 patients with prostate adenocarcinomas (including 12 with clinical stage A, 36 B, 28 C, and 116 stage D), were assayed by polyclonal antiserum-based ELISA for serum PA and PAP levels.

When upper cutoff values of 7.5 ng/ml for PA and 15.5 ng/ml for PAP (both were 95th percentile of BPH values) were used, the combination test resulted in a positive detection rate of 58% for prostate cancer stages A and B, 68% for stage C, and 92% for

stage D, and only 10% for BPH. None of 52 healthy controls and other cancers registered a positive response. In light of the lack of prostate-tumor-specific seromarkers, the use of these two common markers in a simultaneous test may be an effective approach to the immunodiagnosis of prostate cancer. However, recent evidence discussed previously, as obtained from monoclonal-antibody-based reagents, would suggest that PSA assay alone may be as good as PSA and PAP combined.

B. PA, PAP, and Phosphatase Activities

Comparative data on the prognostic value of PA, PAP, and other commonly used enzyme markers associated with the prostate carcinoma have also been reported. Instead of analyzing a limited number of patients and comparing on an individual basis, a double-blind study was performed systematically on a large number of patients and specimens with well-documented clinical information (National Prostatic Cancer Project) [62]. A total of 1,065 serial serum specimens from 79 patients with prostate cancer stages B_2-D_1 (regionally confined disease) and 51 patients with newly diagnosed stage D_2 collected during an 8-year period were analyzed statistically. Forty of the 79 patients with B_2-D_1 disease and 21 of the 51 with D_2 disease presented a clinical progression of disease during follow-up period. In addition to PA and PAP, total acid phosphatase activity, total alkaline phosphatase, and bone isoenzyme of alkaline phosphatase were evaluated for their relative reliability in predicting clinical progression of disease by a series of statistical analyses. For patients with regionally confined disease, only PA and PAP were found to be prognostically important markers, with PA being more significantly reliable than PAP (p values 0.0052 vs. 0.0359). For metastatic prostate cancer, PA ($p < 0.0001$), bone alkaline phosphatase isoenzyme ($p = 0.0007$), and PAP ($p = 0.0206$) were the most reliable markers. Furthermore, after adjustment for the effect of PA, multivariate analyses revealed that no other marker was significantly related to the risk of disease progression. Elevated PA levels were predictive of increased risk even 6 months before disease progression in patients with B_2-D_1 prostate cancer. For all patients, the apparent overall order of prognostic reliability for disease progression was found to be PA > PAP > bone alkaline phosphatase isoenzyme > acid phosphatase > total alkaline phosphatase [62]. This information should be of value as a guide in selecting assays for monitoring disease progression in patients with prostate cancer.

IV. POTENTIAL IMMUNODIAGNOSTIC MARKERS

Several antigenic markers mainly recognized by monoclonal antibodies related to prostate cancer have been reported in recent years. None has reached the stage at which they can be considered for clinical use. Some of these potential markers are briefly described here.

A. KR-P8: Prostate-Specific Marker

KR-P8 is a monoclonal antibody originally prepared against the human prostate cell line PC-3 [63]. By immunostaining, KR-P8 reacted with the glandular epithelium of all specimens of normal, benign hypertrophic, and malignant prostates tested. No reactivity was detected with numerous other human tissues, normal and malignant, including bladder, kidney, and testis, which indicated that KR-P8 recognizes an antigen apparently specific for the prostate. The antigen recognized by the antibody and present in PC-3 cells and in seminal plasma is a glycoprotein with the molecular weight range of 48-75

Kd [64]. The epitope appears to be associated with carbohydrate units, since periodate oxidation resulted in the loss of antigenicity. The potential for using KR-P8 as a marker lies in its secretory characteristics, apparently from the prostate to seminal plasma, and from PC-3 cells to its culture medium. Neither the assay procedure nor its possible presence in patients sera has yet been evaluated. KR-P8 antigen is different from PA biochemically and immunologically; but like PA, it is a secretory product of the prostate, localized in the prostatic epithelia, and apparently prostate specific.

B. 7E11-C5: Prostate-Specfic Marker

A recently reported monoclonal antibody 7E11-C5, generated against the human prostate cell line LNCaP, also exhibited a restricted specificity only to the prostate epithelia [65]. In a panel of 175 human specimens, 7E11-C5 stained only with all 11 specimens of prostate carcinoma, 7 normal, and 7 benign hypertrophic prostate glands. None of the 26 nonprostate tumors nor 120 of the 122 other normal tissue reacted with 7E11-C5. Two of the 14 normal kidney specimens examined were shown to be stained positively. It was interesting that none of 32 cell lines of human normal or neoplastic cells tested was reactive with the antibody. In fact, LNCaP is the only cell line examined to date to react with 7E11-C5. The nature of the antigen remains unknown. Competitive binding inhibition assay revealed the presence of serum antigen in 20 of 43 patients, mostly with advanced prostatic cancer; and in 3 of 66 sera from patients with nonprostatic malignancies. None of the sera from 30 normal blood donors nor 7 with BPH sera was positive. These serum assay results remain to be confirmed by additional investigation with a more sensitive and quantitative assay. It should be noted that PA and KR-P8 are cytoplasmic proteins, while 7E11-C5 antigen is a plasma membrane protein.

C. Turp-27 and PD-41: Prostate-Specific and Prostate Tumor-Specific Markers

Like PA, KR-P8, and 7E11-C5, Turp-27 is a prostate-specific antigen, present in BPH and adenocarcinoma of the prostate and, in reduced concentration, in normal prostate. The monoclonal antibody Turp-27 was generated against a membrane preparation derived from a pool of transurethral resection specimens, which included three BPH and one adenocarcinoma [66]. Therefore, Turp-27 also cannot differentiate BPH from carcinoma. Using a membrane extract prepared identically from a poorly differentiated carcinoma of the prostate, a monoclonal antibody PD-41 was reported most recently [67]. Reactivity of PD-41 appeared to be restricted to prostate carcinoma. PD-41 was not reactive with PA, PAP, normal prostate, BPH, or a variety of normal and nonprostate tumor tissue. Positive immunostain of PD-41 was detected in 83% of poorly differentiated tumor and in only 33% of well-differentiated tumors; thus, PD-41 may be a grade-related and prostate-tumor-specific marker. If the nature of tumor specificity can be confirmed, PD-41 indeed will be one of the most significant prostate tumor markers to become available in recent years, and its clinical potential could be substantial.

D. 83.21 and Other Prostate Tumor-Associated Markers

One of the most extensively characterized monoclonal antibodies associated with prostate cancer is 83.21. This antibody was generated against the human prostate tumor line DU-145 [68]. Initially 83.21 was shown to react with human prostate and bladder

tumor cell lines, and not with 38 other normal or malignant human cell lines. On human prostate tissue sections, reactivity was found in 7 of 11 undifferentiated prostate carcinoma, 4 of 5 poorly differentiated, 1 of 3 moderately differentiated, and none of 6 well-differentiated prostate adenocarcinomas. No reactivity was found with normal prostate or BPH specimens. Reactivity was also shown with one of four bladder tumor specimens, and no stain with two benign bladder tumors or three normal bladder specimens. Of 15 other adenocarcinomas and 30 normal tissues examined, positive stain was found in only 2 normal kidneys at the area of proximal convoluted tubules. A particular feature of this antibody is its reactivity with cytomegalovirus-transformed human embryonic lung cell line. The 83.21 antibody initially was reported to direct against a surface glycoprotein of 180 kD, but recent data revealed that two glycopeptides of 60 kD and 28 kD were isolated from both human prostate tumor and cytomegalovirus-in transformed cells by 83.21 antibody affinity chromatography [69]. 83.21 could be of use in the immunohistopathological characterization of urogenital tumors and in the study of a possible link between cytomegalovirus and transformation of prostate malignancy.

Recent reports have indicated the potential application of anti-Leu-7 (natural killer cells) and anti-Leu 4 (pan T cell) in the immunohistochemical examination of prostate tumor [70,71]. Other potential monoclonal antibodies related to prostate neoplasm include P6.2, αPro-3, αPro-13, αPro-15, Pr-1, and Pr-2 (see Ref. 2 for review). Clinical evaluation of these markers in immunodiagnosis is not yet available.

V. SUMMARY

Immunodiagnosis of prostate cancer is at a more advanced stage than that of most other tumors. Two well-known markers, prostatic acid phosphatase and prostate-specific antigen, have been used in the clinical management of patients. Prostate-specific antigen is a more sensitive and reliable marker than prostatic acid phosphatase. Serum prostate-specific antigen is effective in monitoring disease status, predicting recurrence, and detecting residual disease. Prostate-specific antigen is a tool for the histological differential diagnosis of metastatic carcinomas, especially in the identification of metastatic prostate tumor cells in distant organs and in the differentiation of primary prostate carcinoma from poorly differentiated transitional cell carcinoma of the bladder. Few data on biological function are available. Prostatic acid phosphatase functions as a phosphotyrosyl-protein phosphatase and prostate-specific antigen as a protease. Physiological function in the prostate remains to be elucidated. Several of the prostate-specific and prostate-tumor-associated antigens, as well as a putative prostate tumor-specific antigen, as recognized by monoclonal antibodies are available. Clinical evaluation of these potential markers is not yet available.

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